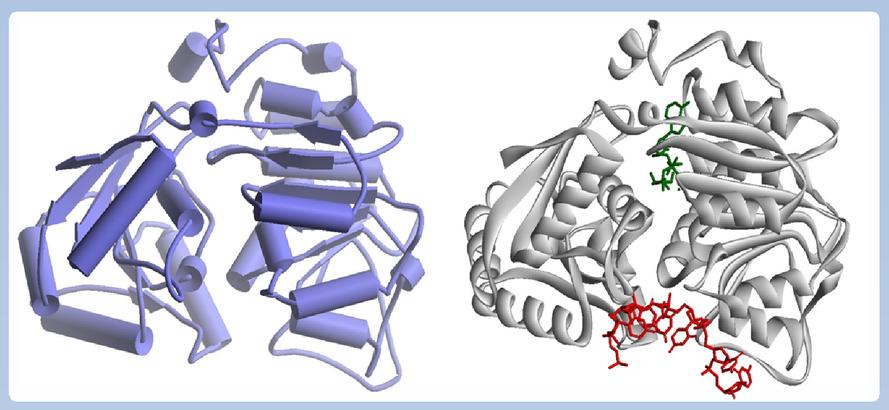
Laboratory of protein biosynthesis

Belyaeva Sofya, Emelyanenko Vera, Minnegalieva Aygul
Supervisers: Mikhaylova Tatyana, Alkalaeva Elena



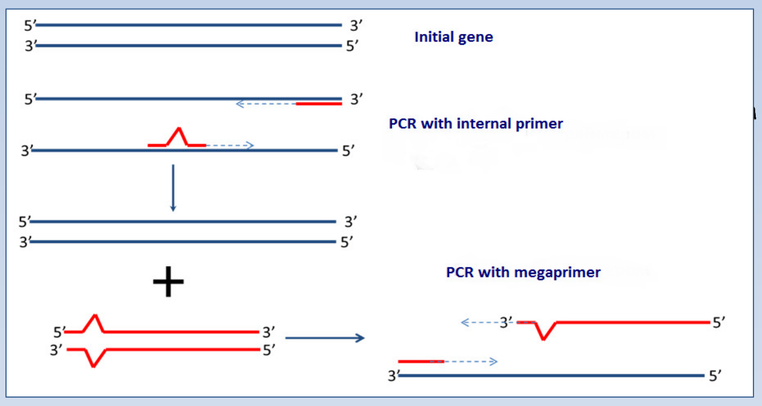
Introduction

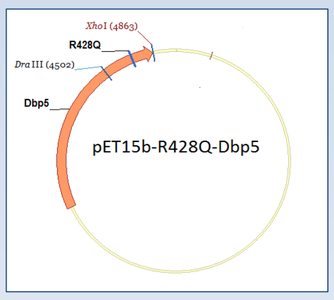
Dbp5 is ATP-dependent DEAD-box RNA helicase essential for mRNA export from the nucleus. Recent study has revealed a novel function for yeast Dbp5 in translation termination (Gross T., 2007). But mechanisms of protein activity in yeast and higher eukaryotes could be different.

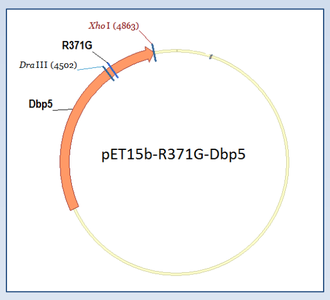
Aim

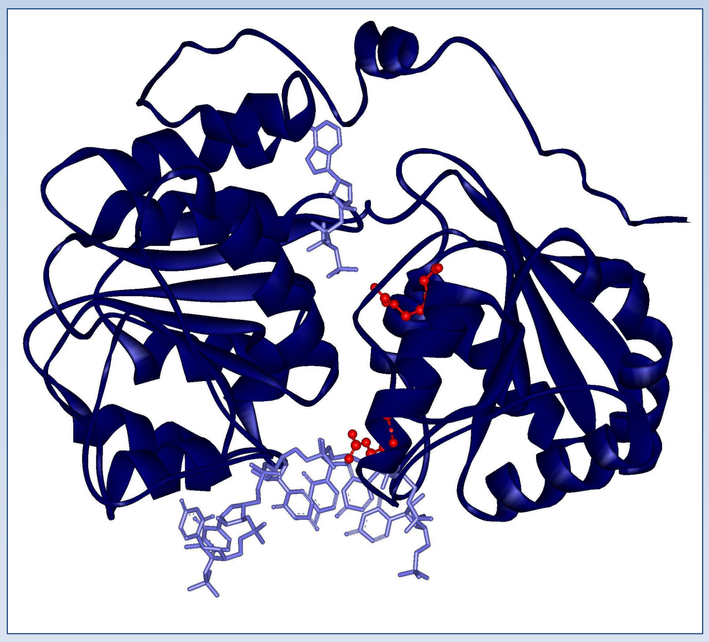
To determine the RNA-factor role of binding with Dbp5 in a regulation of translation termination.

1. The scheme of Dbp5 mutagenesis

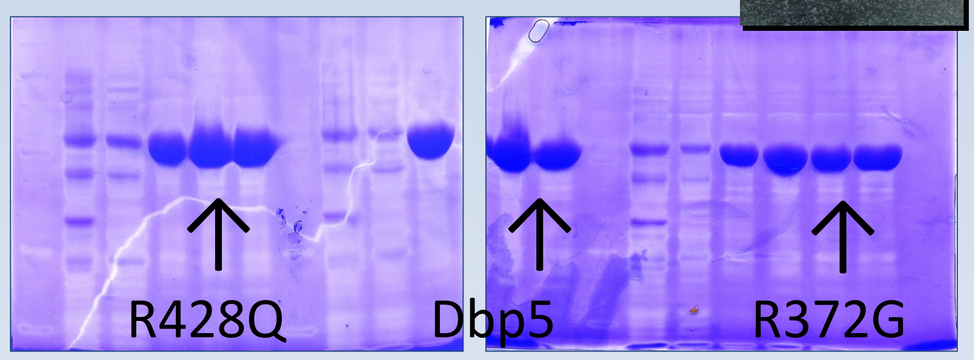






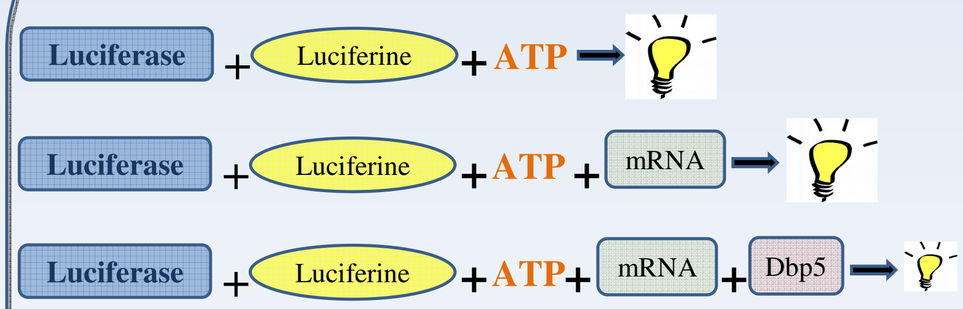


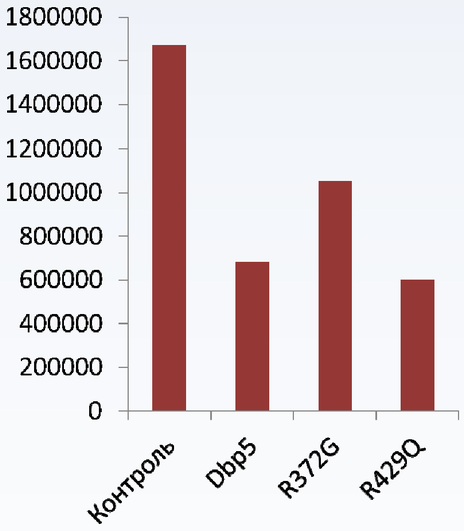
2.Expression of Dbp5 in stamm BL21 E.coli



Dbp5 and its mutant forms were expressed in E.coli and purified with Ni-NTA beads. After that we made PAAG electrophoresis and dialised fractions, containing protein.

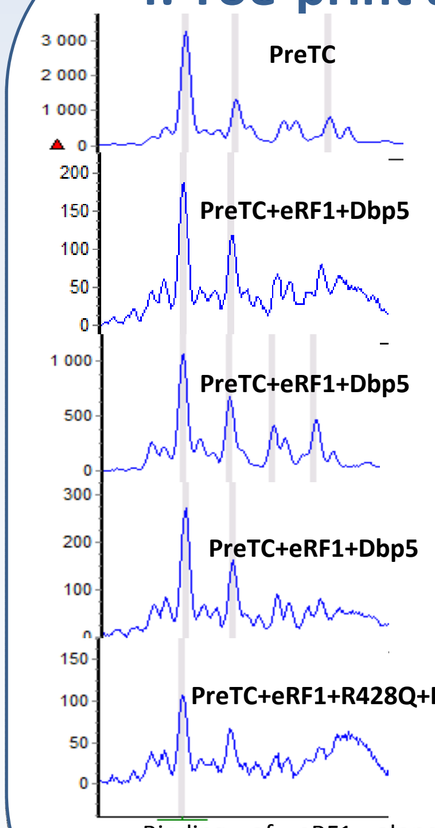
3. Luciferase based assay

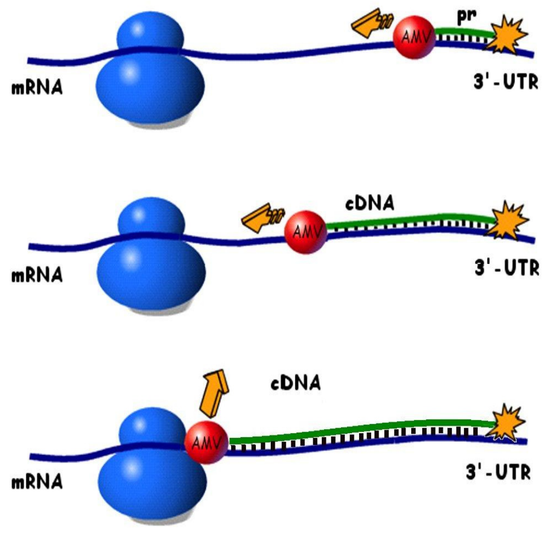




The assay is based on luciferase's requirement for ATP in light production. Thus, if the Dbp5 exerts its helicase activity, the level of ATP in the solution is reduced and, accordingly, the level of luminescence in the sample decreases.

4. Toe-print analysis of [Dbp5-eRF1]





Binding of eRF1 alone to the pretermination complex induces a conformational rearrangement of ribosomal complex characterized by a 2 nt forward shift of the toe-print peak. Mutant proteins do not have an ability to stimulate conformational changes in ribosomes.

Conclusions

1. We have expressed mutant forms of Dbp5 that cannot bind RNAs.
2. Mutant forms of Dbp5 can bind ATPs.
3. For the stimulation of human translation termination Dbp5 requires RNA binding.